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Fully Automated Radiosynthesis of [^{11}C]Guanidines for Cardiac PET Imaging

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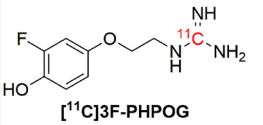
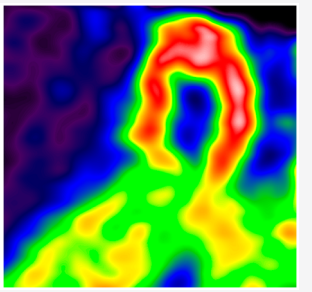
ABSTRACT: Radiolabeled guanidines such as *meta*-iodobenzylguanidine (MIBG) find utility in nuclear medicine as both diagnostic imaging agents and radiotherapeutics and, over the years, numerous methods for incorporating radionuclides into guanidines have been developed. In connection with a project developing new positron emission tomography (PET) radiotracers for cardiac sympathetic nerve density, we had cause to prepare [^{11}C]3F-PHPOG. However, it quickly became apparent that radiolabeling of guanidine scaffolds with carbon-11 has remained challenging, and historical methods lack compatibility with modern automated radiochemistry synthesis platforms and current Good Manufacturing Practice (cGMP) requirements. To address this challenge, we report a new automated method for radiolabeling guanidines with carbon-11. The method was used to prepare a series of [^{11}C]guanidines in good radiochemical yield (8−76% by radio-HPLC) and was found to have broad substrate scope and tolerance of unprotected OH and NH functional groups. The method was used to synthesize [^{11}C]3F-PHPOG for preclinical imaging, and suitability of the radiotracer for preclinical use was demonstrated through preliminary cardiac PET in New Zealand white rabbits which revealed good cardiac uptake and expected retention in the heart.



[^{11}C]3F-PHPOG



KEYWORDS: *sympathetic nervous system, carbon-11, radiochemistry, cardiac PET, positron emission tomography*

There are over 2 million positron emission tomography (PET) scans performed each year in the United States. During a PET scan, patients are injected with a PET tracer (a bioactive molecule tagged with a positron-emitting radionuclide) and scanned in the clinic.[1] Functional information obtained from the PET scan is used to diagnose and stage disease as well as predict and/or monitor response to disease-modifying therapies. Carbon-11 (^{11}C) is a common PET radionuclide because its short half-life (20 min) allows multiple scans to be conducted in series during a single hospital visit, it has excellent imaging properties (99.79% β^+ decay), and multi-Curie amounts of no-carrier-added [^{11}C]CO_2 are readily accessible from small medical cyclotrons via the ^{14}N(p, α)^{11}C nuclear reaction. Reflecting this, considerable effort has been dedicated to development of new methods for incorporating carbon-11 into bioactive molecules[2−4] including recent advances in HCN,[4,5] CO_2,[4,6,7] and CO chemistry.[7,8] Despite these many advances, continued clinical use of ^{11}C-labeled radiotracers (e.g., for cancer imaging[9]), in addition to growing interest in such compounds for drug discovery,[10] necessitates incorporation of carbon-11 into increasingly complex molecules and this process can still be challenging. In particular, certain classes of bioactive and pharmaceutically interesting molecules like guanidines remain notoriously difficult to radiolabel with carbon-11, and such limitations warrant continual development and/or improvement of methods for labeling diverse chemical space with carbon-11.

Radiolabeled guanidines have been widely used in nuclear medicine imaging and therapy for decades. For example, $^{123/131}$I-labeled *meta*-iodobenzylguanidine (MIBG, Figure 1), a

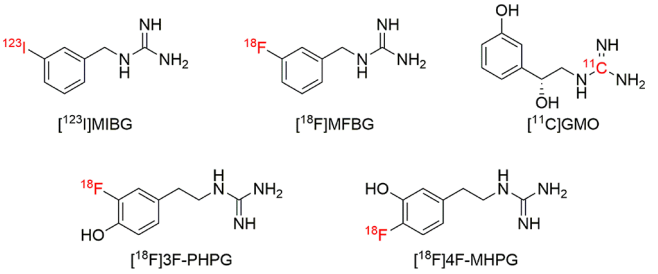


Figure 1. Structures of radiotracers for imaging cardiac sympathetic nerve terminals.

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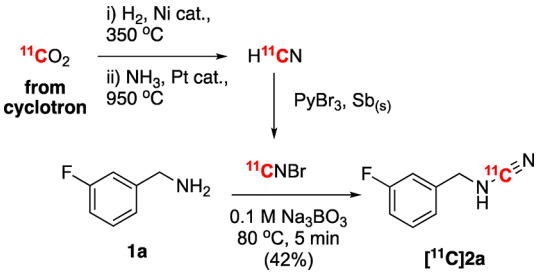
labeled analogue of norepinephrine developed by Wieland and co-workers at our institution,[11] is the best known example and has been used as a radiotracer for imaging and treatment of neuroendocrine tumors as well as noninvasive imaging of cardiac sympathetic nerves. The latter application stems from the fact that, in heart disease, cardiac autonomic dysfunction contributes to morbidity and mortality due to disease-induced alterations in the nervous control of the heart.[12] Such alterations are the result of changes in the outflow of nervous impulses to the parasympathetic and sympathetic branches of the autonomic nervous system arising from central sites in the brain and by regional degeneration of postganglionic parasympathetic and/or sympathetic nerve fibers in the heart. Benzylguanidines have proven to be very useful for scintigraphic imaging studies of cardiac sympathetic innervation, but their uptake rates into cardiac sympathetic neurons are too rapid to allow for robust and reliable compartmental modeling of their kinetics. This, in combination with limitations of scintigraphy, has created much interest in developing guanidine analogues labeled with carbon-11 for PET imaging. Thus, [^{11}C]phenethylguanidine as well as analogues such as *N*-[^{11}C]guanyl-(−)-*m*-octopamine ([^{11}C]GMO),[13,14] ([^{11}C]-*m*-hydroxyphenethylguanidine (MHPG), and ([^{11}C]-*p*-fluoro-*m*-hydroxyphenethylguanidine (4F-MHPG) (Figure 1), have been evaluated as radiotracers with improved kinetics for quantifying cardiac nerve density with PET.[13] Building on these early radiotracers, after bioevaluation of 12 phenenthylguanidines, Raffel and co-workers developed *meta*- and *para*-substituted structural isomers of [^{18}F]fluoro-hydroxyphenethylguanidine ([^{18}F]4F-MHPG and [^{18}F]3F-PHPG) for imaging cardiac sympathetic denervation (Figure 1).[15] First-in-human studies provided robust regional metrics of cardiac sympathetic nerve density.[16]

In an effort to investigate further tuning of the myocardial kinetics of guanidine PET radiotracers to improve quantitative analysis and imaging of neuroendocrine tumors, we were interested in synthesizing hydroxyphenoxyethylguanidine analogues ([^{11}C]-3-fluoro-4-hydroxyphenoxyethylguanidine ([^{11}C]3F-PHPOG, [^{11}C]**3i**), [^{11}C]-1-(3-fluoro-5-hydroxyphenethyl)guanidine ([^{11}C]**3c**), and [^{11}C]-1-(3,4-dihydroxyphenethyl)guanidine ([^{11}C]**3d**)). Since its initial discovery, guanidine has been found in a variety of natural products,[17,18] and many guanidine synthesis methods have been investigated. However, while there has been extensive research devoted to studying guanidino compounds and their syntheses,[17,18] methods for labeling guanidines with carbon-11 remain scarce. Almost 30 years ago, Westerberg and Långström synthesized [^{11}C]MIBG,[19] 1,3-di(2-tolyl)-[^{11}C]-guanidine,[20] and [^{11}C]GG167[21] using [^{11}C]cyanogen bromide ([^{11}C]BrCN).[22] Building on this literature precedent, Raffel recently synthesized a variety of [^{11}C]phenethylguanidines using similar approaches.[13] However, the syntheses of all these radiolabeled guanidines were performed manually using homemade equipment that is no longer particularly amenable to routine clinical production demands in cGMP-compliant PET radiochemistry laboratories. To address this gap in radiochemistry, herein we report a new automated method for radiolabeling guanidines with carbon-11 and use it to prepare a series of [^{11}C]guanidines. Proof-of-concept is demonstrated through preclinical cardiac PET in rabbits with [^{11}C]3F-PHPOG

Our investigation began with optimization of the reaction conditions for the carbon-11 guanylation of a model compound, *meta*-fluorobenzylamine (**1a**) (Scheme 1 and Table 1). A GE TracerLab FX_M automated synthesis module

Scheme 1. Initial [^{11}C]Cyanation of 1a



was used with simple modifications (see the Supporting Information). To accomplish the transformation, [^{11}C]hydrogen cyanide ([^{11}C]HCN) was produced from [^{11}C]CO_2, and then converted to [^{11}C]BrCN using pyridinium perbromide and Sb metal, per previous reports.[5] The tube containing pyridinium perbromide and plug of Sb metal was connected inline between the process panel (where [^{11}C]HCN is generated) and the FX_M synthesis module. The flow of [^{11}C]BrCN was then directly sparged into a solution containing amine precursor **1a** in the reactor of the synthesis module. Initial generation of cyanamide intermediate [^{11}C]**2a** proceeded in 42% RCY (based on HPLC analysis of the crude product as described in the Supporting Information) (Scheme 1). Encouraged by this initial result, we proceeded to investigate the subsequent generation of guanidine product [^{11}C]**3a** (Table 1). However, initial efforts to treat cyanamide intermediate [^{11}C]**2a** with ammonium chloride and generate guanidine product [^{11}C]**3a** resulted in low yield (3%) (Table 1, entry 1). We hypothesized that the second step was slow and inefficient, and that increased temperature, pressure and/or reaction time could improve radiochemical yield. The automated module allows increasing the pressure of the reaction chamber by over pressurizing with nitrogen gas. Overpressurizing for 15 s was insufficient to notably improve yield of [^{11}C]**3a** from [^{11}C]**2a** (Table 1, entry 2). Switching to a saturated ammonium chloride solution and further increasing the time to over pressurize the rector resulted in an improved radiochemical yield of 36−49%, n = 2 (Table 1, entry 3).

Encouraged by these results, we next automated the entire synthesis, including purification of [^{11}C]**3a** by semipreparative HPLC. This resulted in a total synthesis time of 46 min and a noncorrected activity yield (AY) of 31% (Table 1, entry 4). In a previously reported carbon-11 guanylation synthesis by Raffel,[13] NH_4Br was used to form the guanidine instead of NH_4Cl because of the higher solubility of NH_4Br in NH_4OH. However, using our automated method, the use of NH_4Cl (Table 1, entry 4) provided higher yields of [^{11}C]**3a** than NH_4Br (Table 1, entry 5). While developing the semipreparative HPLC method we noted that adjusting pH with acetic acid (Table 1, entry 5) compared to phosphoric acid (Table 1, entry 4) shortened the purification process (and therefore total synthesis time) without impairing separation which is advantageous when working with short-lived carbon-11. Lastly, during the optimization process, we observed that yields of guanidine [^{11}C]**3** improved when freshly prepared NH_4Cl solutions were used.

We next applied the fully automated and optimized carbon-11 guanylation conditions to a series of primary amines (Figure

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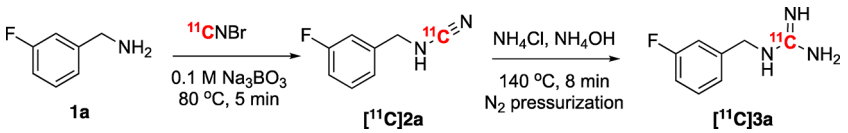
Table 1. Optimization of Guanylation Reaction for Automation



entry	NH_4Cl (%)[a]	temp (°C)	reaction time (min)	overpressure time (s)	yield (%)	total synthesis time (min)
1	20	130	5		3[b]	30
2	20	140	8	15	5[b]	30
3	35	140	8	60	36−49 (n = 2)[b]	30−35
4	35	140	8	60	31[c,d]	46
5	35[e]	140	8	60	22[c,e]	40

[a]Weight to volume percentage of NH_4Cl in 28% NH_4OH solution; [b]Radiochemical yield of [^{11}C]**3a** determined by HPLC analysis of the crude product. [c]AY was calculated from purified product and is noncorrected. [d]130 μL of phosphoric acid was added to adjust the pH of the semipreparative HPLC mobile phase. [e]NH_4Br was used instead of NH_4Cl; 200 μL of acetic acid was added to adjust the pH of the semipreparative HPLC mobile phase.

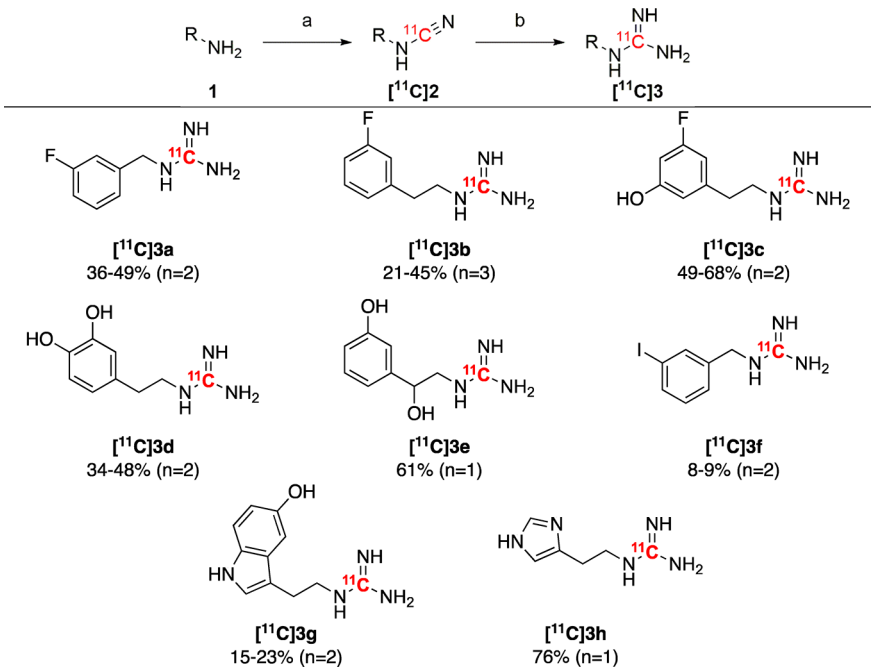


Figure 2. Scope of reaction. Reagents and conditions: (a) [^{11}C]cyanogen bromide, 0.1 M Na_3BO_3, 80 °C, 5 min; (b) 35% NH_4Cl/28% NH_4OH solution, 140 °C, 8 min; RCYs were determined by HPLC analysis of the crude product.

2). All of the amine precursors were commercially available, and the corresponding reference standards were synthesized in our laboratory (see the Supporting Information for details). Seven additional [^{11}C]guanidines were synthesized using the optimized automated synthesis method to explore the scope of the reaction. Most compounds showed modest to good radiochemical yields. Notably, [^{11}C]**3c** was obtained in higher yields (49−68%) than [^{11}C]**3b** (21−45%), demonstrating that the hydroxyl group does not negatively impact the reaction as might be expected in related radiofluorination reactions. This is further illustrated in the synthesis of [^{11}C]**3d** (synthesized from dopamine **1d**) and [^{11}C]**3e**, which both contain two hydroxy groups that did not negatively impact radiochemical yields. These results also suggest that this guanylation reaction is very likely selective for amines. The lower radiochemical yield of [^{11}C]**3f** was not surprising given the lipophilic nature and low solubility of *meta*-iodobenzylamine **1f**, while the basic nitrogen of the indole ring of serotonin (**1g**) was likely a detrimental factor for the cyanogenation step in the preparation of [^{11}C]**3g**. In contrast to the latter example, [^{11}C]**3h** was obtained in the highest radiochemical yield even in the presence of the two nitrogen atoms in the imidazole ring. It is possible that this is simply attributable to the differing electronics of the imidazole ring of histamine **1h** compared to the indole ring of serotonin **1g** but, since a variety of reactions between imidazoles and cyanogen bromide are known,[23] a more sophisticated mechanism involving nucleophilic catalysis cannot be ruled out.

Having successfully optimized the reaction conditions for automation and explored the scope of the reaction, we next shifted our focus to produce injectable doses of radiotracers suitable for preclinical evaluation. Based on previous studies, phenethylguanidines are better substrates for vesicular uptake than their benzylguanidine analogues, and both β-carbon or phenyl ring hydroxyl group substitution leads to greatly extended neuronal retention times.[13] With the future option of ^{18}F-labeling, our first target in mind was [^{11}C]3F-PHPOG ([^{11}C]**3i**). To access this radiotracer, we first prepared 3-

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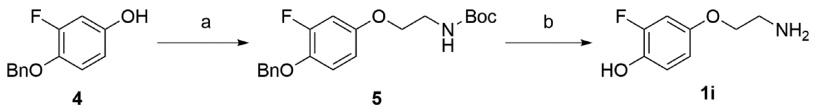
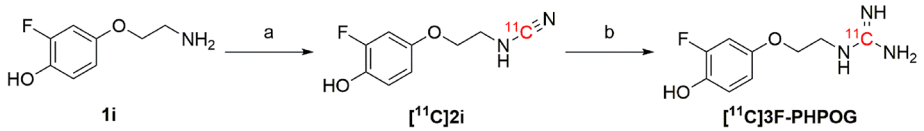
Scheme 2. Synthesis of [^{11}C]3F-PHPOG Precursor 1i[a]



[a]Reagents and conditions: (a) 2-(boc-amino)ethyl bromide, K_2CO_3, DMF, 65 °C, 24 h, 83%; (b) formic acid, concentrated HCl, 80 °C, Ar, 24 h, 63%.

Scheme 3. Synthesis of [^{11}C]3F-PHPOG ([^{11}C]3i)[a]



[a]Reagents and conditions: (a) [^{11}C]cyanogen bromide, 0.1 M Na_3BO_3, 80 °C, 5 min; (b) 35% NH_4Cl/28% NH_4OH solution, 140 °C, 8 min, N_2 pressurization.

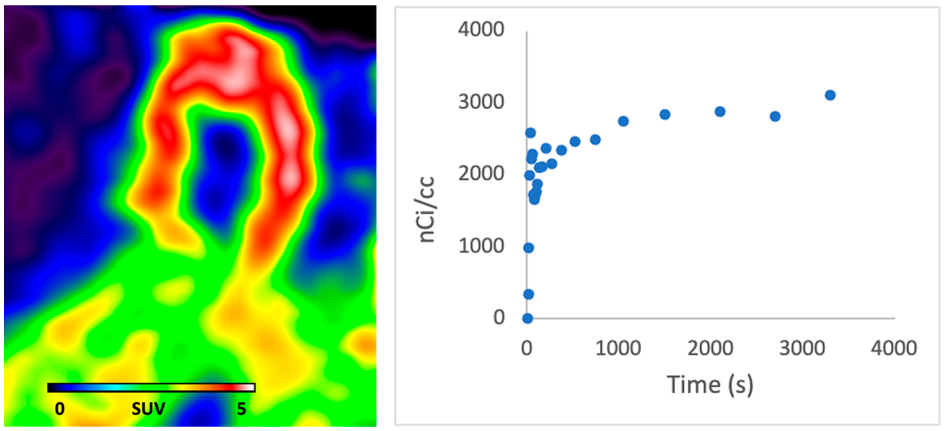


Figure 3. Representative PET image of rabbit heart (0−60 min postinjection of 2.8 ± 0.4 mCi [^{11}C]3F-PHPOG) and associated cardiac time−radioactivity curve (nCi/cc).

fluoro-4-hydroxy-phenoxyethylamine precursor **1i** according to a two-step synthesis (Scheme 2). The synthesis began with the *O*-alkylation of 4-(benzyloxy)-3-fluorophenol **4** with 2-(Boc-amino)ethyl bromide and K_2CO_3 in DMF at 65 °C. Intermediate **5** was subsequently deprotected using 0.1 M formic acid and concentrated HCl at 80 °C to afford [^{11}C]3F-PHPOG precursor **1i**. Using the optimized radiolabeling conditions described above, a full scale synthesis of [^{11}C]3F-PHPOG was developed to prepare doses of radiotracer suitable for preclinical imaging studies (Scheme 3). Briefly, [^{11}C]HCN (~900 mCi) was generated from [^{11}C]CO_2 (~3 Ci) and subsequently converted to [^{11}C]BrCN (540 ± 88mCi). Treatment of **1i** with [^{11}C]BrCN, afforded [^{11}C]**2h**, and treatment with 35% NH_4Cl in NH_4OH at 140 °C for 8 min under nitrogen over pressure yielded [^{11}C]3F-PHPOG ([^{11}C]**3i**). The mixture was quenched with a solution of HPLC mobile phase (10% EtOH in 60 mM NH_4OAc) and acetic acid, and the product was purified by semipreparative HPLC (column: Phenomenex Synergi HydroRP 80A 250 × 10 mm; mobile phase: 10% EtOH in 60 mM NH_4OAc; flow rate: 5.0 mL/min). The purified product did not require reformulation and was simply further diluted with saline for injection, USP, to provide the final injectable dose. The isolated nondecay corrected activity yield of [^{11}C]**3h** was 17 ± 7 mCi (3 ± 1% AY based upon [^{11}C]BrCN), sufficient for preclinical evaluation. The molar activity of the product was 794 ± 220 mCi/μmol and radiochemical purity was >95%. The final injectable dose had a pH of 7, and the synthesis time was 38 ± 2 min (n = 4).

Lastly, after successful production of [^{11}C]3F-PHPOG, we wished to demonstrate the suitability of doses for preclinical imaging and investigated the preliminary imaging properties of the radiotracer in rabbit heart, which is the preferred model of sympathetic neuronal uptake because of similar cardiac physiology to humans. Myocardial imaging was performed by dynamic small animal PET scanning for 60 min in New Zealand white rabbits after administration of [^{11}C]3F-PHPOG (2.8 ± 0.4 mCi, n = 2). A region-of-interest (ROI) was defined for the heart and used to generate the time-radioactivity curve, which revealed both good myocardial uptake of the radiotracer and subsequent retention in the heart (Figure 3). These preliminary results indicate neuronal levels of [^{11}C]3F-PHPOG climb for ≥30 min, which is consistent with previous findings for [^{18}F]3F-PHPG.[16] We also observed high initial uptake in the lung followed by washout throughout the duration of the scan (see Supporting Information). Lung uptake has been observed for guanidine radiotracers previously, and has been shown to vary among species and tracers.[24,25] The imaging properties of [^{11}C]3F-PHPOG will be explored further in future studies beyond the scope of this initial report.

In summary, we report a method for the carbon-11 guanylation of amines that is fully automated and suitable for cGMP manufacture of [^{11}C]guanidines using a commercially

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available radiosynthesis module. A series of amines were tested, including imaging agents for cardiac sympathetic innervation, that demonstrated broad substrate scope and tolerance of unprotected OH and NH functional groups. The optimized method was expanded to new biogenic amines and finally used in the automated production of [^{11}C]3F-PHPOG for preclinical evaluation. Preliminary PET imaging demonstrated good cardiac sympathetic nerve uptake and retention in New Zealand white rabbit heart and showcased use of the automated radiosynthesis to produce PET imaging agents suitable for (pre)clinical applications. Future work will further validate the method for production of clinical doses. We will also evaluate the imaging properties of other [^{11}C]guanidines and expand on the carbon-11 guanylation of new scaffolds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00479.

Full experimental details and copies of NMR spectra and/or HPLC chromatograms for all compounds synthesized; HRMS for standards; procedures for radiochemical syntheses; protocols for animal PET imaging studies (PDF)

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Author Contributions

The manuscript was written through contributions of all authors and all authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

[^{11}C]3F-PHPOG, [^{11}C]-3-fluoro-4-hydroxyphenoxyethylguanidine; [^{11}C]MIBG, *meta*-iodobenzyl-[^{11}C]guanidine; [^{11}C]-GG167, 5-acetylamin-4-[^{11}C]guanidino-2,6-anhydro-3,4,5-trideoxy-$_{D}$-glycero-D-galacto-non-2-enoic acid; [^{123}I]MIBG, *meta*-[^{123}I]iodobenzyl-guanidine; [^{18}F]MFBG, *meta*-[^{18}F]-fluorobenzyl-guanidine; [^{11}C]GMO, *N*-[^{11}C]guanyl-(−)-*m*-octopamine; [^{18}F]3F-PHPG, 3-[^{18}F]fluoro-para-hydroxyphenethylguanidine; [^{18}F]4F-MHPG, 4-[^{18}F]fluoro-meta-hydroxyphenethylguanidine; NET, norepinephrine transporter; VMAT2, vesicular monoamine transporter 2.

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